[19] The State Intellectual Property Office of the People's Republic of China

[51] Int.Cl[7]
C07H 3/04
C07H 1/06

[12] Description of Invention Patent Application Publication

[21] Application No. 01109230.0

[43] Publication Date: August 22, 2001

[11] Publication No. CN 1309131A

[22] Filing Date: Feb 28, 2001

[21] Application No. 01109230.0

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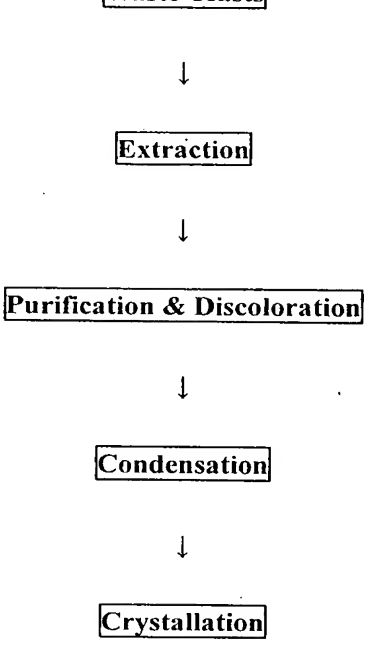
Claims: 1page Description: 5pages Drawings:1page

[54] Title: Process For Producing Trehalose by Using Fermentation Waste

[57] Abstract

A process for producing trehalose by using fermentation waste is provided, wherein said process comprises using fermentation waste such as beer waste yeast slurry, liquor waste yeast slurry, bread active dried yeast dust or high-temperature active brewer's dried yeast dust and the like as raw material and employing reasonable procedures of extraction, purification and crystallation, finally a trehalose product with purity □ 98.5%, capable of being used in fields of vaccines, medicine and biological reagents and the like, is obtained.



ISSN1008-4274

Claims

1. A process for producing trehalose by using fermention waste comprises the following steps:

(1)Extraction:

Select at least one of three solutions of water, ethanol and acetone as an extraction solution, then mix the fermentation waste used as raw material with said extraction solution in a weight ratio of 1:1-5; extract 1-5 times at a temperature of 40-90□and each time lasts 1-5 hours; store the supernatant for later use;

(2)Purification and Discoloration:

Use at least one or more of activated carbon, aluminium oxide, diatomite, bentonite, white clay, porous glass, organic salts, inorganic salts and alginate sodium as purifying discolorant; mix the condensed supernatant with the purifying discolorant in the weight ratio of 100:0.1-5; purify-discolor 1-5 times at a temperature of 30-80□ and each time lasts 0.5-8 hours until the solution becomes colorless; store the purified extraction solution for later use;

(3)Crystallization:

After condensing said purified extraction solution, the sugar concentration reachs 30-70%, then let it crystallize within a crystallization tank; after re-crystallizing and drying, a finished trehalose product of the present invention with purity□ 98.5% is obtained.

2. The process according to claim 1, wherein the fermentation waste in step (1) used as raw material comprise any one of beer waste yeast slurry, liquor waste yeast slurry, active dried yeast dust or high-temperature active brewer's dried yeast dust, or the mixture thereof.
3. The process according to claim 1, wherein the step following step(2) comprises filtrating the purified extraction solution obtained from step(2) through a large-bore resin and crystallizing, then a trehalose product with relatively higher purity is obtained.

Description

Process For Producing Trehalose By Using Fermentation Waste

The present invention relates to recycle of microbiological waste. Specifically, the present invention relates to a process for producing trehalose by using fermentation waste, in particular, the present invention relates to a process for producing high-purity trehalose by using fermentation waste such as beer waste yeast slurry, liquor waste yeast slurry, bread active dried yeast dust or high-temperature active brewer's dried yeast dust and the like.

It is well-known that trehalose is a non-reducing disaccharide consisting of 2 glucose molecules bound by a α, α-1 →1 linkage. Since there is a structure of bipolar axial symmetry in its molecular configuration, trehalose has an extremely strong stability and a low chromogenicity. A large amount of researches have proved that many microorganisms, animals and plants can still maintain their vitality after dehydration under a completely dry condition. The secret lies in the existence of a large amount of trehalose within the body. The researches have further proved that trehalose added in vitro is also capable of stabilizing the structures of cellular membranes, proteins and macromolecules, protecting from staleness. As a stabilizer and protectant of biologically active materials, trehalose has wide-ranging application prospects in such a variety of fields as foodstuffs, nutraceuticals, cosmetics, pharmaceuticals, molecular biological reagents and agriculture.

At present, there are several biological processes for producing trehalose:

1. Fermentation Process: First fermenting and culturing active yeasts and then extracting trehalose therefrom. But the main deficiency is that, as a result of the high cost of producing the yeasts, the price of trehalose extracted and prepared is rather high. Yet trehalose produced by said process has a high purity and can be used as a protectant and a stabilizer of the products such as medical and molecular biological reagents, vaccines and the like. Due to the overly high cost, it is not feasible to apply widely;
2. Enzymatic Convertion Process: Using glucose, sucrose or maltose as medium, and employing the phosphorylase to convert into trehalose; or using starch as substrate to convert into trehalose through the synergistic actions of several enzymes. Employing the enzymatic convertion process for producing trehalose has a relatively low cost. The main deficiency is that the poor product purity makes it unsuitable to apply in such fields as medicine or biological reagents.

Targeting the above deficiencies, the present inventor provides the technical means of the present invention through many years of researches and practices.

The purpose of the present invention is to provide a process for producing trehalose by using fermentation waste. To be specific, by using fermentation waste such as beer waste yeast slurry, liquor waste yeast slurry, bread active dried yeast dust or high-temperature active brewer's dried yeast dust and the like as raw materials and employing reasonable procedures of extraction, purification and crystallation, a high-purity trehalose product capable of being used in such fields as vaccines,

medicines and biological reagents is obtained.

The purpose of the present invention is achieved by a process of using fermentation waste for producing trehalose, wherein the process is characterized in that it comprises the following steps:

(1) Extraction:

Use at least one solution selected from the group consisting of water, ethanol and acetone as a extraction solution, then mix fermentation waste used as raw material with the extraction solution in a weight ratio of 1:1-5. At a temperature of 40-90□, immerse-extract 1-5 times and each time lasts 1-5 hours. Store the supernatant for later use;

(2) Purification and Discoloration:

Use at least one or more of activated carbon, aluminium oxide, diatomite, bentonite, white clay, porous glass, organic salts, inorganic salts and alginate sodium as the purifying discolorant. Mix condensed supernatant with the purifying discolorant in the weight ratio of 100:0.5-5. At a temperature of 30-80□, purify-discolor 1-5 times and each time lasts 0.5-8 hours until the solution becomes colorless. Store the purified extraction solution for later use;

(3) Crystallation:

After condensing the above purified extraction solution, the sugar concentration reachs 30-70%, then let it crystallize within a crystallization tank. Then re-crystallize, dry and obtain the finished product of trehalose of the invention. The purity of trehalose is □ 98.5%;

Wherein said fermentation waste used as raw material in step (1) comprise any one of beer waste yeast slurry, liquor waste yeast slurry, active dried yeast dust or high-temperature active brewer's dried yeast dust, or the mixture thereof; the step following step(2) comprises filtrating the purified extraction solution obtained from step(2) through a large-bore resin and crystallizing, then a trehalose product with relatively higher purity is obtained.

The major advantages of the present invention lie in:

1. Since the present invention utilizes the waste yeasts as the raw materials to extract trehalose, it not only makes a full use of fermentation waste and reduces the environmental pollution, but also lowers greatly the production cost.
2. The present invention adopts any one of beer waste yeast slurry, liquor waste yeast slurry, bread active dried yeast dust or high-temperature active brewer's dried yeast dust, or the mixture thereof to produce high-quality trehalose. Especially the active dried yeast dust is used. Although said yeast cells lose their fermentative activities, the content of trehalose still remains at a high level. The purpose of converting the wastes into the treasures is thus achieved.
3. The present invention makes successful uses of the overall strategy of maintaining the yeast cells as such during the extraction so as to reduce greatly the release of non-sugar substances into the extraction solution. This avoids a large amount of impurities to influence further purification.
4. The present invention solves the problems of separation and purification brought about by the high content of impurities in the waste yeasts, especially those from the oily emulsifiers. At least one or more of active carbon, aluminium oxide,

diatomite, white clay, porous glass, organic salts, inorganic salts or alginate sodium are selected as the purifying discolorant to produce a high-quality trehalose product.

5. The extraction reaction temperature of the present invention is strictly controlled at 30-80°C to consume the least amount of energy to complete the reaction of purifying discoloration so as to lower the production cost of trehalose by a maximal extent.

In short, the process of the present invention not only overcomes the deficiencies of prior art but also utilizes fermentation waste so as to reduce the environmental pollutions caused by said waste material. In addition, the efficacy of producing high-purity trehalose at a low cost and meeting wide-ranging market demands is achieved.

In combination with optimal Examples and Figure, further illustrations are provided below.

Figure 1: Schematic strategy of the procedures according to the present invention

Example 1

Refer to Figure 1. The present invention utilizes as raw material any one fermentation waste selected from the group consisting of beer waste yeast slurry, liquor waste yeast slurry, active dried yeast dust or high-temperature active brewer's dried yeast dust, and obtains a trehalose product with purity □ 98.5% through reasonable procedures of extraction, purification and crystallation. The main steps are as follows:

(1) Extraction:

Utilize at least one of the above-mentioned fermentation wastes or the mixture thereof. Select at least one of three solutions of water, ethanol and acetone as extraction solution, and mix said waste used as raw material with said extraction solution in a weight ratio of 1:1-5. At a temperature of 40-90□, immerse-extract 1-5 times and each time lasts 1-5 hours. Store the supernatant for later use; the remaining residues may be used as the feed proteins after drying.

(2) Purification and Discoloration:

Since the present invention utilizes fermentation waste as raw material, there are many impurities including proteins and oily emulsifiers. Therefore at least one or more of activated carbon, aluminium oxide, diatomite, bentonite, white clay, porous glass, organic salts, inorganic salts and alginate sodium are used as the purifying discolorant. After concentration, the supernatant is mixed with the purifying discolorant in the weight ratio of 100:0.5-5. At a temperature of 30-80□, purify-discolor 1-5 times and each time lasts 0.5-8 hours until the solution becomes colorless. Store the purifying extraction solution for later uses;

(3) Crystallation:

Condense the purified extraction solution until the sugar concentration reachs 30-70%. Place it in a crystallation tank to crystallize. Then wash and dry the crystals to obtain a finished trehalose product with purity □ 98.5%. Since the crystallation procedure is one being commonly employed, there is no need to re-elaborate;

For the purified extraction solution obtained from Step (2), it is possible to crystallize directly or after filtrating through a large-bore resin, and a trehalose product with

purity □ 98.5% is obtained.

Example 2

Weight 100kg yeast dust and add 200kg mixture of water and ethanol. Extract 3 times at 50°C and each lasts 3 hours. Combine all the extraction supernatants. The residues may be used as feed.

After concentrating the supernatant into 100kg, add 1.5kg each of active carbon and aluminium oxide as the purifying discolorant. Purify-discolor 3 times at 30-80°C and each time lasts 3 hours. Separate and condense the supernatant till the sugar concentration up to 50%, then filter and wash the crystals to prepare a trehalose product of the present invention. Upon testing, the purity of said trehalose is 99.1%.

Example 3

Weight 100kg waste yeasts and add 300kg water for extraction. Extract 4 times at 70°C and each time lasts 1.5 hours. Combine all the supernatants and use the residues as the feed. After condensing the supernatant into 100kg, add 0.5kg each of diatomite and white clay as the purifying discolorant. Purify-discolor at 60°C for 2 hours. Then add 1kg each of active carbon and porous glass, purify-discolor 2 times and each time lasts 3 hours. Separate the supernatant and run through a large-bore resin ionic exchange column to obtain a clear solution. Then condense to make the sugar concentration to 60%. Crystallize and then filtrate and wash the crystals. After drying, a high-purity trehalose product of the present invention is obtained. Upon testing, the purity of said trehalose is 99.5%.

Example 4

Weight 100kg beer waste yeast slurry and add 200kg water for extraction. Extract at 65□ 2 times and each time lasts 2 hours. Combine all the supernatants and the residues may be used as the feed. After condensing the supernatant into 100kg, add 0.8kg each of diatomite and aluminium oxide as the purifying discolorant. Purify at 65□ for 2 hours and then add 0.6kg each of active carbon and alginate sodium. Purify-discolor 2 times and each time lasts 3 hours. Separate the supernatant and run through a large-bore resin ionic exchange column to obtain a clear solution. Then condense to make the sugar concentration to 65%. Crystallize and then filtrate and wash the crystals. After drying, a trehalose product of the present invention is obtained. Upon testing, the purity of said trehalose is 99.0%.

Drawings

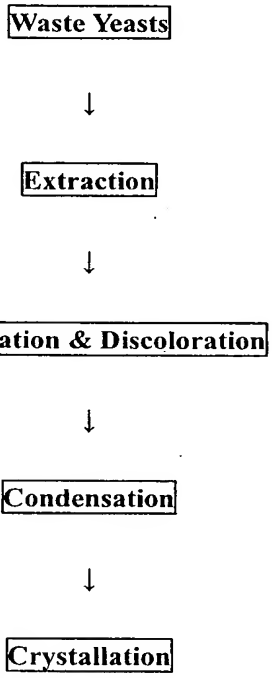


FIG 1

[19]中华人民共和国国家知识产权局

[51]Int. Cl[7]
C07H 3/04
C07H 1/06

[12]发明专利申请公开说明书

[21]申请号 01109230.0

[43]公开日 2001年8月22日

[11]公开号 CN 1309131A

[22]申请日 2001.2.28 [21]申请号 01109230.0

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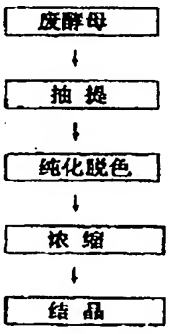
权利要求书1页 说明书5页 附图页数1页

[54]发明名称 利用发酵废弃物生产海藻糖的方法

[57]摘要

一种利用发酵废弃物生产海藻糖的方法，即利用啤酒废酵母泥、白酒废醪 母泥、面包活性干酵母粉尘或高温酿酒活性干酵母粉尘等发酵废弃物为原料，通过合理的提取、纯化和结晶工艺，获得纯度为≥98.5%的可用于疫苗、医药 和生物试剂等领域的海藻糖产品。



ISSN1008-4274

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权 利 要 求 书

1、一种利用发酵废弃物生产海藻糖的方法，其特征在于：它包括如下的生产工艺：

（1）抽提：

选择水、乙醇和丙酮三种溶剂中的至少一种溶液作为提取液，将发酵废弃物原料与提取液按 1：1-5 的重量配比，在 40—90℃的条件下，浸提 1—5 次，每次 1—5 小时，留取上清液备用；

（2）纯化脱色：

使用活性碳、三氧化二铝、硅藻土、皂土、白土、多孔玻璃、有机盐、无机盐和海藻酸钠其中的至少一种或几种混合使用作为纯化脱色剂，该上清液浓缩后与纯化脱色剂按 100：0.1-5 的重量配比，在 30—80℃的条件下，进行纯化脱色 1—5 次，每次 0.5—8 小时，至液体无色，为纯化提取液，备用；

（3）结晶：

将上述纯化提取液浓缩后，使糖浓度达到 30—70%，置结晶罐中结晶，然后再洗晶、烤干，得到本发明的海藻糖成品，其海藻糖的纯度为≥98.5%。

2、如权利要求 1 所述的方法，其特征在于：所述步骤（1）的发酵废弃物原料包括啤酒废酵母泥、白酒废酵母泥、活性干酵母粉尘或高温酿酒活性干酵母粉尘中的任意一种或其混合物。

3、如权利要求 1 所述的方法，其特征在于：所述步骤（2）制造的纯化提取液，包括经大孔树脂过滤后结晶，得到较高纯度的海藻糖产品。

01.02.28

说　明　书

利用发酵废弃物生产海藻糖的方法

本发明涉及废弃微生物再利用的技术领域，特指一种利用发酵废弃物生产海藻糖的方法，即利用啤酒废酵母泥、白酒废酵母泥、面包活性干酵母粉尘或高温酿酒活性干酵母粉尘等发酵废弃物，制备高纯度的海藻糖的工艺方法。

众所周知，海藻糖（trehalose）是由两个葡萄糖分子经α，α-1→1 键接的非还原性双糖，由于其分子构象存在双折轴向对称结构，因而海藻糖具极强的稳定性和低呈色性。大量研究证明：许多微生物和动植物在完全干燥失水后仍可保持生命活性，其奥妙就在于体内含有大量海藻糖。研究还证明：外加的海藻糖同样具有稳定细胞膜、蛋白质和生物大分子结构及抗逆保鲜作用。作为生物活性物质的稳定剂和保护剂，海藻糖在食品、保健品、化妆品、制药、分子生物学试剂和农业等领域有着广阔的应用前景。

目前生物法生产海藻糖主要有以下几种方法：

1、发酵法：其是先发酵培养活性酵母，再从中提取海藻糖。其主要缺陷在于：由于生产酵母的成本高，使提取制备的海藻糖价格昂贵，但发酵法生产的海藻糖纯度高，可作为医药、分子生物学试剂、疫苗等产品的保护剂、稳定剂。由于成本过高，不适合广泛地推广应用；

2、酶促转化法：其是以葡萄糖、蔗糖或麦芽糖为介质，通过磷酸化酶反应转化成海藻糖；或以淀粉为底物通过几种酶协同作用转化成海藻糖。用酶促转化法生产海藻糖，成本相对较低，其主要缺陷在于：产品纯度较差，不适合医药或生物制剂等领域的应用。

针对上述缺陷，本发明人经过长期地研究和实践，创造出本发明的技术方

案.

本发明的目的在于提供一种利用发酵废弃物生产海藻糖的方法，即利用啤酒废酵母泥、白酒废酵母泥、面包活性干酵母粉尘或高温酿酒活性干酵母粉尘等发酵废弃物为原料，通过合理的提取、纯化和结晶工艺，获得高纯度的可用于疫苗、医药和生物试剂等领域的海藻糖产品。

本发明的目的是这样实现的：一种利用发酵废弃物生产海藻糖的方法，其特征在于：它包括如下的生产工艺：

（1）抽提：

选择水、乙醇和丙酮三种溶剂中的至少一种溶液作为提取液，将发酵废弃物原料与提取液按 1：1-5 的重量配比，在 40—90℃的条件下，浸提 1—5 次，每次 1—5 小时，留取上清液备用；

（2）纯化脱色：

使用活性碳、三氧化二铝、硅藻土、皂土、白土、多孔玻璃、有机盐、无机盐和海藻酸钠其中的至少一种或几种混合使用作为纯化脱色剂，该上清液浓缩后与纯化脱色剂按 100：0.5-5 的重量配比，在 30—80℃的条件下，进行纯化脱色 1—5 次，每次 0.5—8 小时，至液体无色，为纯化提取液，备用；

（3）结晶：

将上述纯化提取液浓缩后，使糖浓度达到 30—70%，置结晶罐中结晶，然后再洗晶、烤干，得到本发明的海藻糖成品，其海藻糖的纯度为≥98.5%。

上述步骤（1）所述的发酵废弃物原料包括啤酒废酵母泥、白酒废酵母泥、活性干酵母粉尘或高温酿酒活性干酵母粉尘中的任意一种或其混合物。上述步骤（2）制造的纯化提取液，包括经大孔树脂过滤后结晶，得到较高纯度的海

藻糖产品。

本发明的主要优点是:

1、由于本发明利用废弃酵母为原料提取海藻糖，不仅充分利用了发酵废弃物，减少了环境污染，而且大大降低了生产成本。

2、本发明选用啤酒废酵母泥、白酒废酵母泥、面包活性干酵母粉尘或高温酿酒活性干酵母粉尘中的任意一种和其混合物生产出高品质的海藻糖。特别是利用了活性干酵母粉尘，这些酵母细胞的发酵活性虽已丧失，而海藻糖含量仍然较高的特点，达到变废为宝的目的。

3、本发明成功地运用了在提取过程中保持酵母细胞的整体性策略，因而大大减少了非糖物质向提取液中释放，避免了因杂质多，影响进一步纯化。

4、本发明针对废弃酵母中杂质含量高，特别是油性乳化剂等杂质给分离纯化带来的困难，从活性碳、三氧化二铝、硅藻土、白土、多孔玻璃、有机盐、无机盐或海藻酸钠中选择至少一种或几种混合作为纯化脱色剂，生产出高品质的海藻糖产品。

5、本发明的提取反应温度严格控制在 30—80℃，达到在最低耗能下，完成纯化脱色反应，以最大限度地降低生产海藻糖的成本。

总之，本发明的方法，不仅克服了现有技术的缺陷，而且利用了发酵废弃物，减少了这些废弃物对环境的污染，并且达到了以低成本生产高纯度海藻糖，满足市场广泛需求的功效。

下面结合较佳实施例和附图进一步说明。

图 1 为本发明的制造工艺示意图。

实施例 1

参阅图 1，本发明是利用啤酒废酵母泥、白酒废酵母泥、活性干酵母粉尘或高温酿酒活性干酵母粉尘中的任意一种发酵废弃物为原料，经合理的提取、纯化和结晶工艺，获得纯度≥98.5%的海藻糖产品。其主要生产工艺如下：

（1）、抽提：

利用上述发酵废弃物的至少一种或其混合物，选择水、乙醇和丙酮三种溶剂中的至少一种溶液作为提取液，废弃物原料与提取液按 1：1-5 的重量配比，在 40—90℃的条件下，浸提 1—5 次，每次 1—5 小时，留取上清液备用；剩余的残渣烤干后可作为饲料蛋白。

（2）纯化脱色：

由于本发明使用的原料为发酵废弃物，含杂质较多，主要杂质包括蛋白、油性乳化剂等，故使用活性碳、三氧化二铝、硅藻土、皂土、白土、多孔玻璃、有机盐、无机盐和海藻酸钠等其中的至少一种或几种混合使用作为纯化脱色剂，上清液浓缩后与纯化脱色剂按 100：0.5-5 的重量配比，在 30—80℃的条件下，进行纯化脱色 1—5 次，每次 0.5—8 小时，至液体无色，为纯化提取液，备用；

（3）结晶：

将纯化提取液浓缩，使糖浓度达到 30—70%，置于结晶罐中结晶，然后再洗晶、烤干，得到本发明的海藻糖成品，其海藻糖的纯度为≥98.5%。由于结晶方法为常用的方法，故不重述；

上述步骤（2）制造的纯化提取液，可直接结晶或经大孔树脂过滤后结晶，得到较高纯度≥98.5%的海藻糖产品。

实施例 2

称取酵母粉尘 100kg，加入 200kg 水与乙醇的混合物，在 50℃抽提 3 次，每次 3 小时，合并几次抽提上清液。残渣可作为饲料。

上清液浓缩成 100kg 后，加入活性碳和三氧化二铝各 1.5kg 作为纯化脱色剂，在 30—80℃纯化脱色 3 次，每次 3 小时，分离上清液，浓缩使糖浓度达 50%后结晶，然后滤晶、洗晶，烤干后，制成本发明的海藻糖产品，经测试，其海藻糖的纯度为 99.1%。

实施例 3

称取废弃酵母 100kg，加入 300kg 的水抽提，在 70℃抽提 4 次，每次 1.5 小时，合并几次上清液，残渣可作为饲料。上清液浓缩成 100kg 后，加入硅藻土和白土各 0.5kg 作为纯化脱色剂，在 60℃纯化脱色 2 小时，再加入活性碳和多孔玻璃各 1 kg，纯化脱色 2 次，每次 3 小时，分离上清液经过大孔树脂离子交换柱得清液，再浓缩至糖浓度为 60%后，结晶，然后滤晶、洗晶，烤干后，制成本发明的高纯度的海藻糖产品，经测试，其海藻糖的纯度为 99.5%。

实施例 4

称取啤酒废酵母泥 100kg，加入 200kg 的水提取，在 65℃抽提 2 次，每次 2 小时，合并几次上清液，残渣可作为饲料。上清液浓缩成 100kg 后，加入硅藻土和三氧化二铝各 0.8kg 作为纯化脱色剂，在 65℃纯化 2 小时，再加入活性碳和海藻酸钠各 0.6 kg，纯化脱色 2 次，每次 3 小时，分离上清液过大孔树脂离子交换柱得清液，再浓缩至糖浓度为 65%后，结晶，然后滤晶、洗晶，烤干后，制成本发明的海藻糖产品，经测试，其海藻糖的纯度为 99.0%。

说 明 书 附 图

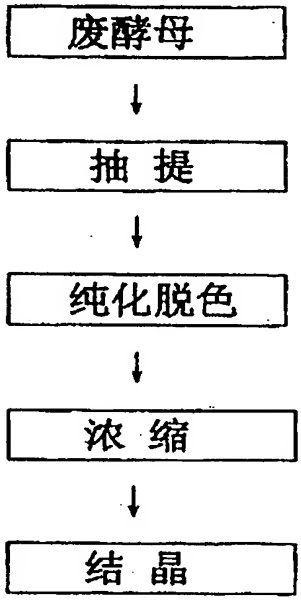

图 1